The Role of Blood Pressure and Aldosterone in the Production of Hemorrhagic Stroke in Captopril-Treated Hypertensive Rats

Andrew B. MacLeod, BSc; Sudesh Vasdev, PhD; John S. Smeda, PhD

From the Division of Basic Medical Sciences, Memorial University, St John's, Newfoundland, Canada.

Correspondence and reprint requests to Dr J.S. Smeda, Division of Basic Medical Sciences, Rm H4354, Memorial University, Health Science Centre, St John's, Newfoundland, Canada A1B 3V6.
Abstract

Background and Purpose We tested the hypothesis that the lowering of plasma aldosterone levels contributed to the antistroke effects of captopril treatment in Wistar Kyoto stroke-prone spontaneously hypertensive rats (SHRSP).

Methods The suppression of plasma aldosterone by captopril treatment (50 mg · kg⁻¹ · d⁻¹) was prevented by the subcutaneous infusion of aldosterone into captopril-treated SHRSP. We studied the effect this had on blood pressure (BP) and stroke development.

Results SHRSP fed a Japanese-style diet containing 4% NaCl developed hypertension and a 100% mortality associated with intracerebral hemorrhage by 14 weeks of age. Captopril treatment from 6 weeks of age did not lower the BP but increased survival past 35 weeks of age. Hydralazine treatment (40 to 80 mg/L of drinking water) lowered BP in SHRSP but was less effective than captopril in retarding stroke. Plasma aldosterone levels were elevated with age in SHRSP after 10 weeks and were higher in poststroke versus prestroke SHRSP. Captopril treatment suppressed plasma aldosterone. When we elevated plasma aldosterone in captopril-treated SHRSP to levels between those present in untreated pre- and poststroke SHRSP, the ability of captopril to retard stroke development was negated. The effects of aldosterone were mimicked by deoxycorticosterone (40 mg/kg, SC2 times/wk) but not by dexamethasone (0.1 mg · kg⁻¹ · d⁻¹, SC). Spironolactone treatment (20 mg · kg⁻¹ · d⁻¹, SC) of SHRSP reduced BP but had little effect on stroke development.

Conclusions Elevations in plasma aldosterone enhance stroke development within captopril-treated SHRSP through mechanisms that do not involve stimulation of mineralocorticoid receptors or the enhancement of hypertension. The antistroke effects of captopril treatment may be partially mediated through the suppression of plasma aldosterone.

Key Words: aldosterone • angiotensins • cerebral hemorrhage • captopril • rats
Introduction

Studies involving Wistar Kyoto SHRSP have shown that both plasma renin levels and activity increase with age and that plasma renin activity and Ang II levels are elevated in SHRSP with established hypertension when compared with normotensive WKY. The renin angiotensin system responds to dietary NaCl in a unique manner in SHRSP. Whereas elevations in dietary sodium suppress plasma renin activity in normal rats, renin activity and stroke development are enhanced in SHRSP. Treatment of salt-loaded SHRSP with ACEIs, or the Ang II receptor antagonist losartan, produces a long-term delay in the onset of stroke development. Despite the fact that plasma renin activity and Ang II levels are elevated in these animals, the latter drugs are ineffective antihypertensive agents when administered to salt-loaded SHRSP, and the antistroke effects of the drugs occur under conditions where only a small decrease in blood pressure is observed.

The mechanisms by which ACEIs and losartan inhibit stroke development in SHRSP have not been elucidated. Speculation has centered around the possibility that the small decrease in blood pressure often produced during ACEI treatment and/or the prevention of short-term elevations in blood pressure could play a role in the prevention of stroke development. Other researchers have speculated that ACEIs produce an antistroke effect independent of a change in blood pressure. It has been suggested that such treatment may protect against vascular damage produced by the elevations in plasma Ang II. A reduction in neutrophil chemoattraction to vascular endothelium, decreased vascular smooth muscle hypertrophy and hyperplasia, decreased vascular permeability, and a reduction in the incidence of cerebrovascular fibrinoid necrosis have all been suggested as possible beneficial mechanisms that could act to retard stroke development in response to the lowering of Ang II levels through ACEI or losartan treatment. To date little consideration has been given to the possibility that elevations in plasma aldosterone may play a role in stroke development in SHRSP. Plasma aldosterone increases in a parallel manner to renin activity with increasing age in SHRSP; and during established hypertension, the levels of plasma aldosterone and Ang II are both four to six times the levels present in normotensive rats.
In the present study we assessed the hypothesis that the antistroke effects of ACEI treatment with captopril are, in part, mediated by a lowering of plasma aldosterone levels. We measured plasma aldosterone levels before and after stroke development in untreated SHRSP and SHRSP subjected to captopril treatment. Subsequently, we replaced the levels of plasma aldosterone suppressed by captopril treatment through a subcutaneous infusion of aldosterone into captopril–treated SHRSP and determined the effect that this had on stroke development. We also tested the ability of the mineralocorticoid deoxycorticosterone to duplicate the effects of aldosterone. In the design of the study we anticipated that the above manipulations could modify blood pressure within SHRSP. To assess the potential influence of altered blood pressure during treatment, hydralazine was used to lower blood pressure to levels below those observed during captopril treatment, and the glucocorticoid hypertensive agent dexamethasone was administered to raise blood pressure in captopril–treated SHRSP. We reasoned that if captopril treatment produced an antistroke effect by lowering blood pressure, then other more effective antihypertensive agents should be able to duplicate or exceed the antistroke effects of captopril. Conversely, elevations in blood pressure through the use of hypertensive drugs should nullify the effects of captopril treatment. Finally, we assessed the ability of the type I aldosterone receptor antagonist spironolactone to inhibit stroke development in SHRSP.

Methods

Animals

Only male SHRSP were used in the study. These were taken from a colony maintained at Memorial University of Newfoundland (St John’s, Newfoundland, Canada). The rats were fed a Japanese-style diet containing 4% NaCl (Zeigler Brothers). Individual treatments were initiated within the 6th week (wk) of life and complied with the institutional guidelines of Memorial University of Newfoundland and the Canadian Council on Animal Care.

Treatments

Groups of SHRSP were subjected to the following treatments: (1) captopril, 50 mg · kg⁻¹ · d⁻¹, administered orally in the drinking water (drinking rates were monitored and the concentration of the drug in the drinking water was modified to achieve the correct dose); (2) hydralazine, 20, 40, 60, 80, and 100 mg/L of drinking water; (3) aldosterone, 0.66 µg/hr administered subcutaneously by implanted osmotic...
pumps (Alzet) within polyethylene glycol vehicle (300 molecular weight) infused at a rate of 0.5 µL/h (pumps were implanted under anesthesia produced by 10 mg xylazine and 50 mg ketamine per kg, IM); (4) dexamethasone, 0.1 mg · kg⁻¹ · d⁻¹ injected subcutaneously within olive oil at 0.1 mL/100 g; (5) deoxycorticosterone (acetate salt), 40 mg/kg injected subcutaneously 2 times/wk within olive oil at 0.1 mL/100 g; and (6) spironolactone, 20 mg · kg⁻¹ · d⁻¹ injected subcutaneously within olive oil at 0.1 mL/100 g. In addition, three groups of SHRSP were treated with combined treatments of *captopril* plus aldosterone, *captopril* plus dexamethasone, and *captopril* plus deoxycorticosterone at the dosages and modes of administration described above. Cotreatment with aldosterone, deoxycorticosterone, or dexamethasone was started on average 3 days after the initiation of *captopril* treatment. Control groups consisted of untreated SHRSP, SHRSP subjected to polyethylene glycol infusion by osmotic pump at 0.5 µL/h (volume used to infuse aldosterone), and SHRSP injected subcutaneously with olive oil at 0.1 mL · 100 g⁻¹ · d⁻¹ (volume of vehicle used to inject spironolactone and dexamethasone). In addition, *captopril*-treated SHRSP were infused with aldosterone (polyethylene glycol) or injected with dexamethasone/spironolactone (olive oil) vehicles equivalent to the amounts used in the combined treatment studies described above. All the above drugs were purchased from Sigma Chemical Co.

**Monitoring of Stroke Development**

The rats were monitored daily for the occurrence of stroke. The symptoms associated with stroke development within SHRSP have been previously described. Initially, SHRSP develop convulsive repetitive forearm movement followed by inappropriate posture during which the rat sits with its legs hyperextended in a "kangaroo-type" posture. The latter symptom was often associated with lethargy and poor grooming. There is typically a 1.5-week period between the onset of the first behavioral symptoms of stroke and death. Some animals died abruptly after the first behavioral symptoms of stroke. Other animals were killed at a point when death was likely to occur within a day. These latter animals were anesthetized, and a blood sample was taken by cardiac puncture. The brain was removed from all animals and fixed with 84 mmol/L PO₄ buffer containing 4% formaldehyde and 1% glutaraldehyde, pH 7.4. In most cases, the presence of intracerebral hemorrhage could be clearly seen within one or both cerebral hemispheres. In cases in which this did not occur, the entire brain, including the brain stem, was sectioned and examined histologically for the presence of intracerebral hemorrhage.
Physiological Measurements
The systolic blood pressure of all the rats was measured weekly using a tail-cuff compression method (IITC Life Science Instruments model 29, pulse/pressure amplifier). Radioimmunoassay techniques were used to measure plasma aldosterone levels within untreated SHRSP and in SHRSP treated with captopril in the presence and absence of aldosterone infusion. These were performed by the Memorial University, Health Science Centre Renal Diagnostic Laboratory (St John’s, Newfoundland, Canada) by qualified personnel that perform these assays on a routine basis. The assays were performed using the Coat-A-Count radioimmunoassay kit (Diagnostic Products Corp). The personnel analyzing plasma aldosterone were blinded to the identity of the samples.

Statistical Analysis
Alterations of blood pressure with age were analyzed using a general linear model of multivariant ANOVA. The effects of varying treatments on blood pressures were determined with respect to age over common ages between treatment groups. Mortality with age was assessed using a Mann-Whitney rank order test. Plasma aldosterone levels between multiple groups were analyzed using a one-way ANOVA to determine whether between-group differences existed. Subsequently, Student’s t test compensated for multiple comparisons with the Bonferroni method was used to determine which groups differed from each other. Changes in plasma aldosterone levels with age were analyzed using linear regression analysis coupled with the determination of a Pearson product of correlation (r value). Results were considered significant at P<.05. All results are expressed as the mean±one standard error measurement.

Results
Figure 1A and 1B, respectively, shows the alterations in blood pressure and mortality with age in control and captopril-treated SHRSP. Control SHRSP developed stroke and either died or were euthanized at a stage of near death between 10 to 14 weeks of age. Treatment with captopril 50 mg · kg⁻¹ · d⁻¹ dramatically increased the survival of the SHRSP. At 18 weeks of age none of the 12 SHRSP treated with captopril demonstrated behavioral evidence of stroke. To determine that intracerebral hemorrhage was absent in these animals, 5 of the 12 SHRSP treated with captopril were randomly selected and sampled between 18 to 20
weeks of age. No histological evidence of intracerebral hemorrhage was observed. The remaining 7 SHRSP were allowed to live and continued with captopril treatment up to a maximum of 39 weeks of age after which the experiment was terminated. Between 34 and 39 weeks of age, 3 of 7 SHRSP exhibited behavioral signs of stroke and either died or were euthanized near death. All exhibited histological evidence of intracerebral hemorrhage. At 39 weeks of age the 4 surviving SHRSP were sampled. These animals appeared healthy and lacked behavioral symptoms of stroke; however, 2 of the 4 SHRSP exhibited histological evidence of intracerebral hemorrhage. The above results suggested that captopril treatment not only retarded the onset of intracerebral hemorrhage in SHRSP but may have also enhanced survival after intracerebral hemorrhage had developed. When assessed over the life span of the control SHRSP, captopril treatment did not significantly alter the blood pressure of SHRSP.

Figure 1. Alterations in systolic blood pressure (A) and mortality (B) associated with stroke in untreated control SHRSP and SHRSP treated with captopril or hydralazine. Over the life span of the control SHRSP, captopril treatment (50 mg · kg⁻¹ · d⁻¹, orally) did not significantly alter blood pressure in SHRSP and all SHRSP survived up to 34 weeks of age. The experiment was terminated at 38 weeks of age (**) with 4 captopril-treated SHRSP surviving. These animals lacked behavioral symptoms of stroke but 2 of 4 SHRSP exhibited small intracerebral hemorrhages. To study the effects of reduced blood pressure on stroke development, SHRSP were treated with hydralazine (40 to 100 mg/L dw). Mortality from stroke was studied in SHRSP that responded to hydralazine treatment by exhibiting average systolic blood pressures <220 mm Hg from 10 weeks of age to death. Despite the presence of lower blood pressures in the hydralazine-treated group, the onset of mortality associated with stroke occurred at an earlier age than that observed in captopril-treated SHRSP. Statistics in panel A: ANOVA, over the life span of A, A vs B is NS, for A vs C A<0.001; over the life span of C, for B vs C A<0.001; in panel B: Mann-Whitney, for A vs B A<0.001, for A vs C A<0.001, for B vs C A<0.01. (In A, n=11; B, n=7; C, n=7 SHRSP.) dw indicates in drinking water.
Previous studies involving the treatment of SHRSP with ACEIs or losartan have demonstrated a statistically significant depression in blood pressure during the early phases of hypertension development (9 to 13 weeks). It has been suggested that a small suppression of blood pressure during this critical period of blood pressure development may produce a profound effect in retarding subsequent stroke development in SHRSP. Although we did not observe a significant suppression of hypertension with captopril treatment, we further explored this possibility. SHRSP were subjected to antihypertensive treatment by introducing hydralazine into the drinking water at doses of 20, 40, 60, and 100 mg/L. The antihypertensive effects of hydralazine varied among animals. It was not uncommon for the animals to initially exhibit a suppression in blood pressure only to have their blood pressure re-elevate to levels that were present in control animals. Since the objective of the study was to achieve an antihypertensive effect and not to study the effects of hydralazine treatment per se, mortality was only studied in hydralazine-treated SHRSP that exhibited average weekly systolic blood pressures of less than 220 mm Hg from 10 weeks of age, a time when hypertension is fully established, to the time of death. Three SHRSP treated with 40 mg hydralazine/L in their drinking water, one 60 mg/L–treated SHRSP, two 80 mg/L–treated SHRSP, and one 100 mg/L–treated SHRSP met the latter criteria, and the blood pressure and mortality data pertaining to this group of animals are plotted in Fig 1. As shown in Figure 1A, when compared over common life spans, hydralazine treatment significantly suppressed blood pressure development in the SHRSP when compared with nontreated control SHRSP and captopril-treated SHRSP. Such treatment retarded mortality associated with stroke development within SHRSP by about 7 weeks (Fig 1B). Despite the presence of higher blood pressures, captopril treatment was considerably more effective than hydralazine treatment in retarding the onset of mortality associated with stroke. These results would suggest that captopril treatment retards the onset of mortality associated with stroke through mechanisms that are independent of its potential ability to lower blood pressure.

In subsequent experiments we attempted to determine whether the ability of captopril treatment to retard stroke development in SHRSP was related to the suppression of plasma aldosterone levels. We observed that within prestroke SHRSP, plasma aldosterone levels tended to rise with age in SHRSP after 10 weeks of age (Fig 2). Also, as shown in Fig 3, SHRSP (10.5 to 13.5 weeks of age) that developed stroke had, on average, more than five times the levels of plasma
aldosterone that was present in comparably aged prestroke SHRSP. Captopril treatment suppressed plasma aldosterone to 25% of the levels observed in untreated prestroke SHRSP and to 4% of the levels present in untreated poststroke SHRSP. To investigate the influence of plasma aldosterone levels on stroke development, SHRSP were treated with captopril from 6 weeks of age. After 3 days of captopril treatment aldosterone was continuously infused subcutaneously at 0.66 µg/h into captopril-treated animals by implanted osmotic pumps. Through preliminary studies we determined that this rate of aldosterone infusion would elevate plasma aldosterone levels in 10- to 13-week-old captopril-treated SHRSP to levels between those present in comparably aged nontreated pre- and poststroke SHRSP. As shown in Fig 4, SHRSP that received captopril treatment plus aldosterone infusion had blood pressures that did not significantly differ from those present in SHRSP that received no treatment when these groups were compared over common life spans. When compared over common life spans captopril plus aldosterone–treated SHRSP did have higher blood pressures than SHRSP treated only with captopril. However, at ages past those where a 100% mortality occurred in SHRSP treated with captopril plus aldosterone, SHRSP treated with captopril alone achieved blood pressures equal to or higher than those of the former group and still survived. As indicated in Fig 4B, the infusion of aldosterone into SHRSP treated with captopril prevented the ability of captopril treatment to retard the onset of stroke development. An analysis of plasma aldosterone levels of the SHRSP treated with captopril plus aldosterone infusion (Fig 3) indicated levels that were less than those of untreated SHRSP that developed stroke and greater than those present in comparably aged prestroke SHRSP. The data were consistent with the hypothesis that the ability of captopril to retard the onset of stroke development in SHRSP may be produced by a lowering of plasma aldosterone levels.

Figure 2. Alterations in plasma aldosterone with age in untreated SHRSP aged 9.5 to 12.5 weeks before the development of hemorrhagic stroke. The results suggest that plasma aldosterone may inappropriately rise with age in SHRSP (r=.62, P<.01, n=15 SHRSP).
Figure 3. Plasma aldosterone levels in untreated SHRSP and captopril-treated SHRSP with and without aldosterone supplement. The results show that plasma aldosterone values were higher in untreated poststroke vs prestroke SHRSP. Captopril treatment (50 mg · kg⁻¹ · d⁻¹, orally) significantly reduced plasma aldosterone, whereas the subcutaneous infusion of aldosterone (0.66 µg/h) into captopril-treated SHRSP raised plasma aldosterone to levels between those present in pre- and poststroke SHRSP. One-way ANOVA of groups 1 to 5, \( P < 0.001 \); 1 vs 3, 1 vs 4, 1 vs 5, 2 vs 4, 2 vs 5, 3 vs 4, and 3 vs 5 were all significantly different at \( P < 0.05 \); Student's t test compensated for multiple comparisons using Bonferroni correction (for group 1, n=15 SHRSP; group 2, n=5; group 3, n=15; group 4, n=5; and group 5, n=5).

Figure 4. Alterations in systolic blood pressure (A) and mortality associated with stroke (B) in untreated control and captopril-treated SHRSP in the presence or absence of aldosterone infusion. The infusion of aldosterone (0.66 µg/h, SC) into captopril-treated (50 mg · kg⁻¹ · d⁻¹, orally) SHRSP raised plasma aldosterone to levels between those present in untreated pre- and poststroke SHRSP (see Fig 3○). The infusion of aldosterone into captopril-treated SHRSP reversed the antistroke effects of captopril treatment. Statistics in panel 4A: ANOVA, over the life span of A, A vs B is NS; over life span of C, A vs C is NS, for B vs C \( P < 0.01 \); in panel B: Mann-Whitney, A vs C is NS, for B vs C \( P < 0.001 \) (for A, n=11; B, n=7; and C, n=9).

In other experiments, we tested the ability of deoxycorticosterone and dexamethasone to mimic the effects of aldosterone within captopril-treated
SHRSP. Deoxycorticosterone is a mineralocorticoid with properties similar to aldosterone, whereas dexamethasone is a glucocorticoid that lacks mineralocorticoid activity. Both drugs have the ability to elevate blood pressure. SHRSP were treated with captopril from 6 weeks of age, and deoxycorticosterone (40 mg/kg 2 times/wk) or dexamethasone (0.1 mg · kg\(^{-1}\) · d\(^{-1}\)) was injected into the animals. As shown in Fig 5A, when compared over common life spans the injection of deoxycorticosterone or dexamethasone did not significantly elevate the blood pressure of SHRSP over that present in control animals, but deoxycorticosterone and dexamethasone injections both elevated the blood pressure of captopril-treated animals. Like aldosterone infusion, the injection of deoxycorticosterone into captopril-treated SHRSP prevented captopril treatment from retarding mortality associated with stroke development, whereas dexamethasone did not (Fig 5B). None of the 6 rats treated with captopril plus dexamethasone died or exhibited symptoms related to stroke development (Fig 5B). The captopril plus dexamethasone-treated SHRSP were sampled between 24 and 30 weeks of age. None of the rats exhibited behavioral symptoms of stroke; however, histological examination revealed the presence of intracerebral hemorrhage in 2 of the 6 animals. The conclusions reached were that despite the ability of dexamethasone to elevate blood pressure in captopril-treated SHRSP, the glucocorticoid did not reverse the effects of captopril treatment on mortality to the extent of that observed during combined captopril plus mineralocorticoid treatment with either deoxycorticosterone or aldosterone. The experiments also suggested that the small elevations in blood pressure observed during captopril plus mineralocorticoid treatment, compared with that of captopril treatment alone, likely did not contribute to the reversal of the antistroke effects of captopril treatment.

**Figure 5.** Alterations in systolic blood pressure (A) and mortality associated with hemorrhagic stroke (B) within untreated control SHRSP and SHRSP treated with captopril in the presence or absence of subcutaneous co-treatment with dexamethasone or deoxycorticosterone. The co-treatment of captopril-treated SHRSP with deoxycorticosterone (40 mg · kg\(^{-1}\) · d\(^{-1}\) 2 times/wk SC) but not with dexamethasone (0.1 mg/kg) prevented captopril treatment from retarding the onset of hemorrhagic stroke. None of the captopril-treated SHRSP...
We also tested the individual effects of aldosterone, dexamethasone, and deoxycorticosterone on blood pressure and mortality associated with stroke within SHRSP not treated with captopril. The results of this experiment are shown in Fig 6. The blood pressures of SHRSP subjected to aldosterone infusion, deoxycorticosterone, or dexamethasone injections did not significantly differ from untreated control SHRSP when compared over corresponding life spans (Fig 6A). Aldosterone infusion and deoxycorticosterone injections accelerated the onset of mortality associated with stroke on average by 2.2 to 2.5 weeks when compared with untreated SHRSP (Fig 6B). The injection of dexamethasone did not significantly alter stroke development in SHRSP.

In other experiments, we tested the ability of spironolactone to duplicate the effects of captopril treatment. Canrenone, a metabolic by-product of spironolactone, acts as a competitive antagonist for the type I aldosterone...
The role of blood pressure and aldosterone in the production of hem...

receptor present within the renal tubule and other cells. Spironolactone was injected subcutaneously into SHRSP at 20 mg · kg⁻¹ · d⁻¹ from 6 weeks of age. As shown in Fig 7A, spironolactone treatment significantly suppressed blood pressure development within SHRSP over the life span of the control SHRSP. Such treatment slightly delayed mortality associated with stroke within SHRSP (Fig 7B).

Figure 7. Alterations in systolic blood pressure (A) and mortality associated with stroke (B) in untreated control and spironolactone-treated SHRSP.

Spironolactone (20 mg · kg⁻¹ · d⁻¹, SC) treatment of SHRSP significantly reduced blood pressure but produced only a small increase in the life span of the animals. Spironolactone treatment did not duplicate the effects of captopril treatment. In panel A: ANOVA, over life span of A, A vs B P<.001; in panel B: Mann-Whitney, A vs B P<.05 (for A, n=11; B, n=5).

The potential effects of the infusion/injection vehicles on stroke development within SHRSP were also studied. The injection vehicle for the administration of spironolactone, dexamethasone, or deoxycorticosterone was olive oil that was administered at a volume of 0.1 mL/100 g body wt. SHRSP injected daily with the above vehicle died at an average age of 12.2±0.3 weeks (range, 11.2 to 13.0 weeks; n=4). SHRSP infused with polyethylene glycol (aldosterone vehicle) at 0.5 µL/h by implanted osmotic pumps from 6 weeks of age died at an average age of 13.6±0.5 weeks (range, 13.0 to 15.0 weeks). When mortality profiles were compared with those of untreated SHRSP (average age of death, 12.4±0.36 weeks; range 10.3 to 13.7 weeks; n=11), no significant differences were observed (Mann-Whitney rank order test). In other experiments, captopril-treated SHRSP (50 mg · kg⁻¹ · d⁻¹) were injected with olive oil (the deoxycorticosterone vehicle, n=4) or infused with polyethylene glycol (the aldosterone vehicle) by osmotic pumps (n=4). None of these animals developed stroke. The latter experiments were terminated when SHRSP reached an average age of 17 weeks, which surpassed the age at which a 100% mortality occurred within captopril-treated SHRSP receiving either aldosterone...
infusion (0.66 µg/h, see Fig 4B) or deoxycorticosterone injections (40 mg/kg 2 times/wk, see Fig 5B). When the alterations in blood pressure with age were statistically analyzed over comparable life spans, no significant differences were observed between untreated SHRSP, SHRSP treated with captopril alone, and SHRSP injected with olive oil or infused with polyethylene glycol vehicle in the presence or absence of captopril treatment (group effect of treatments, P=.436). It was concluded that the acceleration in stroke development observed in SHRSP treated with deoxycorticosterone or aldosterone, the inhibition of the antistroke effects of captopril treatment observed during cotreatment with aldosterone or deoxycorticosterone, and the antihypertensive effects observed during spironolactone treatment could not be attributed to the vehicle used.

Discussion

The key finding of the present study was the observation that the reconstitution of plasma aldosterone levels to levels observed before the suppression of this hormone by captopril treatment negated the antistroke effects of captopril treatment. Plasma aldosterone levels were elevated with age in SHRSP after 10 weeks of age and were about five times higher in SHRSP that developed stroke when compared with similarly aged SHRSP that had not yet developed stroke. This is a remarkable elevation in plasma aldosterone levels when one considers that the prestroke animals already had twice the plasma aldosterone levels typically observed within normotensive 5- to 25-week-old WKY. Captopril treatment of SHRSP suppressed plasma aldosterone to levels comparable to those previously reported in WKY and prevented mortality associated with stroke up to 34 weeks of age. The reinfusion of aldosterone into captopril-treated SHRSP to achieve levels between those present in pre- and poststroke nontreated SHRSP totally negated the effects of captopril treatment and caused mortality to be comparable to that observed in untreated SHRSP. The effects of aldosterone could be duplicated by injecting captopril-treated SHRSP with the mineralocorticoid deoxycorticosterone. These results suggest that under conditions in which Ang II levels should be suppressed by captopril, elevations in plasma aldosterone represent a significant risk factor for stroke development within SHRSP.

One of our concerns was that aldosterone was infused into SHRSP from an early
age (6.5 weeks of age). Since the osmotic pumps delivering aldosterone do so at a constant delivery (0.66 µg aldosterone/h), younger and smaller SHRSP might have been subjected to higher plasma aldosterone levels than those observed in 10- to 13-week-old captopril plus aldosterone-treated SHRSP. Recently we have started treating SHRSP with captopril from 10 weeks of age, a point at which we have shown in the present study that plasma aldosterone levels rise with age. We observed that captopril treatment (50 mg · kg⁻¹ · d⁻¹) totally prevented mortality associated with stroke up to the termination of the experiment (24 weeks of age), whereas the co-treatment of captopril-treated SHRSP with aldosterone starting at 10 weeks of age (3 days after the initiation of captopril treatment) largely negated the antistroke effects of captopril treatment (captopril plus aldosterone-treated SHRSP mean age of death, 16.5±0.8 weeks, n=6; untreated SHRSP, mean age of death, 14.0±0.4 weeks, n=8; captopril-treated SHRSP, no death up to 24 weeks of age, n=7; unpublished results, 1997). This would suggest that elevations in aldosterone within prestroke SHRSP from 10 weeks of age enhance stroke development with SHRSP.

It is difficult to explain the antistroke effects of captopril treatment (50 mg · kg⁻¹ · d⁻¹) on the basis of an antihypertensive effect or to explain the ability of aldosterone to reverse the antistroke effects of captopril on the basis of the very small increases in blood pressure observed. When blood pressure was compared over the life span of untreated SHRSP, captopril treatment did not produce a statistically significant depression in blood pressure. On the other hand, hydralazine treatment significantly depressed blood pressure within SHRSP but only extended the life span of SHRSP by about one third of that produced by captopril treatment. The highest average blood pressures within the study were observed in captopril-treated SHRSP co-treated with dexamethasone. At the termination of the experiment (24 weeks of age) they had systolic blood pressures that in many cases exceeded 300 mm Hg. These blood pressures were equal to or higher than the highest blood pressures present within captopril-treated SHRSP co-treated with aldosterone or deoxycorticosterone, yet none of these animals died from stroke at 24 weeks, whereas a 50% mortality associated with stroke was observed before 13 weeks of age in captopril-treated SHRSP supplemented with either aldosterone or deoxycorticosterone. The above experiments support the argument that the ability of aldosterone or deoxycorticosterone to reverse the effects of captopril treatment occurs through mechanisms other than a slight elevation in blood pressure produced by the above mineralocorticoid.
Other studies have indicated that the inhibition of stroke development as a result of captopril and other ACEI treatments was associated with the inhibition of cerebrovascular fibrinoid necrosis development. It is possible that the occurrence of vascular fibrinoid necrosis could weaken the cerebral vascular wall causing such sites to burst or leak vascular fluids and that the prevention of the development of this vascular pathology might act to inhibit stroke development. However, some studies of salt-loaded SHRSP have noted the occurrence of cerebral hemorrhage in SHRSP under conditions where histological examination indicated the virtual absence of fibrinoid necrosis within the cerebrovasculature. Our most recent studies indicate that captopril treatment (50 mg·kg⁻¹·d⁻¹) of SHRSP prolongs their life span (by 5 to 9 weeks) even when treatment is started 1 to 5 days after the initial signs of stroke, at a time when cerebrovascular fibrinoid necrosis might be present (unpublished results, 1997). This would suggest that captopril treatment can increase the life span of SHRSP via mechanisms beyond the inhibition of cerebrovascular fibrinoid necrosis development.

At the present time we are unclear as to the mechanisms through which elevations in plasma aldosterone induce stroke development within captopril-treated SHRSP. An important finding in the present study was the observation that treatment of SHRSP with spironolactone (20 mg·kg⁻¹·d⁻¹) could not duplicate the effects of captopril treatment. Spironolactone is quickly metabolized to canrenone, and both spironolactone and canrenone are potent competitive antagonists of the type 1 mineralocorticoid receptor. Within distal segments of the rat nephron and proximal regions of the collecting ducts, aldosterone binds to the intracellular type 1 receptor within the epithelium and initiates an increase in Na⁺ permeability and the de novo synthesis of Na⁺/K⁺ ATPase, both of which facilitate the uptake of Na⁺ from the urine into the blood. Initially we felt that elevations in plasma aldosterone may aggravate sodium loading within SHRSP by enhancing the uptake of this ion from the urine. Since these animals were already being fed a high salt diet, which is a risk factor for stroke development, such a modification could further aggravate stroke. However, the uptake of Na⁺ from the urine should have been inhibited not only by lowering plasma aldosterone levels with captopril treatment but also by spironolactone treatment. Despite the fact that the dose of spironolactone used in the present study (20 mg·kg⁻¹·d⁻¹, SC) was about four times the per kilogram daily oral dose used to treat Conn’s syndrome patients, we considered the possibility that the dose was too small or delivered too infrequently (once a day). In this regard, canrenone has a half-life of 10 to 35 hours in humans.
and the dose of spironolactone used did produce an antihypertensive effect in SHRSP suggesting that the drug was physiologically active. In recent experiments that we have performed, higher doses of spironolactone (150 mg · kg⁻¹ · d⁻¹, SC) initiated at 10 weeks of age did not alter stroke development in SHRSP (unpublished results, 1997). Hence, we believe that the ineffectiveness of spironolactone was not due to insufficient quantities of the drug being administered. The conclusion we have reached is that the ability of aldosterone to accelerate stroke development in captopril-treated SHRSP likely occurs through a mechanism that does not involve activation of the type 1 mineralocorticoid receptors. A possible speculative mechanism by which this could occur is through the stimulation of nongenomic plasma membrane aldosterone receptor. These receptors have been recently discovered and their actions characterized on the surface of vascular smooth muscles, endothelium, and human leukocytes. Within vascular smooth muscle, aldosterone has been shown to produce a rapid rise in free intracellular Ca²⁺, a phospholipase C-mediated increase in diacylglycerol production and protein kinase C activation, an increase in Na⁺ efflux thought to be mediated by enhanced Na⁺/H⁺ porter activity, and a stimulation of Na⁺/K⁺ ATPase. These effects were observed within seconds to 4 minutes after the application of doses of aldosterone or deoxycorticosterone <1 nmol/L. The effects could not be inhibited by canrenone and could not be duplicated by high (0.1 to 1 µmol/L) levels of hydrocortisosterone or dexamethasone. How such mechanisms might act to facilitate stroke development within captopril-treated SHRSP is unclear. An important mechanism involved in promoting cerebral blood-flow autoregulation is the occurrence of cerebral vascular constriction in response to elevated pressure. The loss of such function could promote over perfusion of the cerebral vasculature during hypertension and thus lead to the production of intracerebral hemorrhage. The occurrence of smooth muscle depolarization and the activation of protein kinase C are both thought to be important second messenger events linking the sensing of pressure to the promotion of vascular constriction. One could speculate that elevations in plasma aldosterone, perhaps through the stimulation of the nongenomic aldosterone receptor, might alter smooth muscle ionic fluxes, protein kinase C function, and/or produce cytotoxic effects that could alter cerebral blood-flow autoregulation in a manner conducive to the development of stroke under hypertensive conditions. In this regard, previous studies have indicated that the middle cerebral arteries of prestroke SHRSP lose their ability to constrict to pressure after 10 weeks of age, at about the time plasma aldosterone levels elevate. More recent studies have
indicated that such function is preserved in captopril-treated SHRSP but not captopril plus aldosterone—or captopril plus deoxycorticosterone-supplemented SHRSP.\(^\text{30}\)

It is tempting to extend the interpretation of the results of this study to suggest that elevations in plasma aldosterone levels, independent of any direct effect of Ang II, are responsible for stroke development in SHRSP. The results we have presented are consistent with the above hypothesis but such a conclusion is premature. Support for the above view would be strengthened by the observation that stroke development is retarded in adrenalectomized SHRSP subjected to aldosterone infusions that maintain plasma aldosterone below the levels observed in prestroke SHRSP. In such an instance, the independence of an Ang II effect with respect to stroke development would dictate that the coinfusion of Ang II should not alter the onset of stroke development. Until such experiments are performed we can only conclude that by elevating plasma aldosterone levels during captopril treatment you inhibit the ability of captopril to act as an antistroke agent.

Selected Abbreviations and Acronyms

ACEIs = Ang I-to-Ang II converting-enzyme inhibitors
Ang I/II = angiotensin I or II
SHRSP = stroke-prone spontaneously hypertensive rats
WKY = Wistar Kyoto control rats

Acknowledgments

This study was supported by a grant from the Heart and Stroke Foundation of Newfoundland and Labrador.

Received October 17, 1996; revision received May 15, 1997; accepted June 11, 1997.

References

1. Gahnem F, von Lutterotti N, Camargo MJF, Laragh JH, Sealey JE. Angiotensinogen dependency of blood pressure in two...
The Role of Blood Pressure and Aldosterone in the Production of Hem...

Methods

Results

Discussion

References


**This article has been cited by other articles:**

- **Am. J. Physiol. Endocrinology and Metabolism**

- **Therapeutic Advances in Cardiovascular Disease**
Enalapril Prevents Imminent and Reduces Manifest Cerebral Edema in Stroke-Prone Hypertensive Rats • Editorial Comment
Stroke, August 1, 1998; 29(8): 1671 - 1678.
[Abstract] [Full Text] [PDF]

Mineralocorticoid Blockade Reduces Vascular Injury in Stroke-Prone Hypertensive Rats
[Abstract] [Full Text] [PDF]